Geographic variation in pentane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers)

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ABSTRACT

Populations of *Helianthus annuus*, ranging from eastern Oklahoma to coastal southern California, were sampled and the yields of total hydrocarbons (HC) from leaves determined. The highest yielding populations were in the Texas Panhandle (6.0 - 7.99%) and the lowest yields were in Camp Verde, AZ, NM mountains, Redland, OR, and San Diego, CA. Medium-high yields were found in northern UT and southern ID. Four populations near Waco, TX had large yield differences ranging from 3.6 to 6.2%. Some native populations were contaminated by germplasm from cultivated sunflowers and these populations had very low yields (2.6 - 3.6%). Population variability in HC yields varied geographically and also between nearby populations, suggesting the micro-habitat environments are important as well as limited genetic population size. The frequency distribution (329 individuals) ranged from 1.0 to 12.63% yield and showed a skewed, normal distribution, with a tail towards highest yielding plants. The mean was 5.33%, with the top 5% being larger than 8.7% yield. A very low correlation (r=0.18) was found between leaf size biomass and % yield implying an opportunity to select for high yields and high biomass concurrently. Published on-line www.phytologia.org *Phytologia 99(1): 1-10 (Jan 19, 2017).* ISSN 030319430.

KEY WORDS: *Helianthus annuus,* Sunflower, geographic variation in leaf hydrocarbon yields.

Adams and Seiler (1984) surveyed 39 taxa of sunflowers for their cyclohexane (hydrocarbon) and methanol (resins) concentrations. The highest cyclohexane (bio-crude) yielding taxa were *H. agrestis*, an annual, Bradenton, FL (7.38%) and *H. annuus*, Winton, OK (7.09%). Adams et al. (1986) screened 614 taxa from the western US for their hydrocarbon (hexane soluble) and resin (methanol soluble) yields. They reported 2 plants of *H. annuus* from Idaho with 8.71% and 9.39% hydrocarbon yields.

Seiler, Carr and Bagby (1991) reported on 28 *Helianthus* taxa for their yields of oil, polyphenols, protein and rubber. The rubber was found to be of lower molecular weight than *Hevea* rubber, but still appeared to be useful as a plasticizing additive and for coatings inside pipes and containers. Yields of natural rubber has recently been reported for *H. annuus* (Pearson et al. (2010a) that ranged from 0.9% to 1.7% rubber in cultivated sunflower cultivars (Fig. 4, Pearson et al. 2010b).

There does not appear to be any information on geographic variation in the yields of hydrocarbons for *H. annuus*. The purpose of this report is to present new information on geographic variation of the yields of pentane extractable hydrocarbons in native, annual sunflower. This is continuation of our research on sunflowers (Adams and TeBeest, 2016; Adams, et al. 2016).

MATERIALS AND METHODS

Population locations - see Appendix I.

The lowest growing, non-yellowed, 8 mature leaves were collected at stage R 5.1-5.3 (Figure 1) when the first flower head opened with mature rays. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

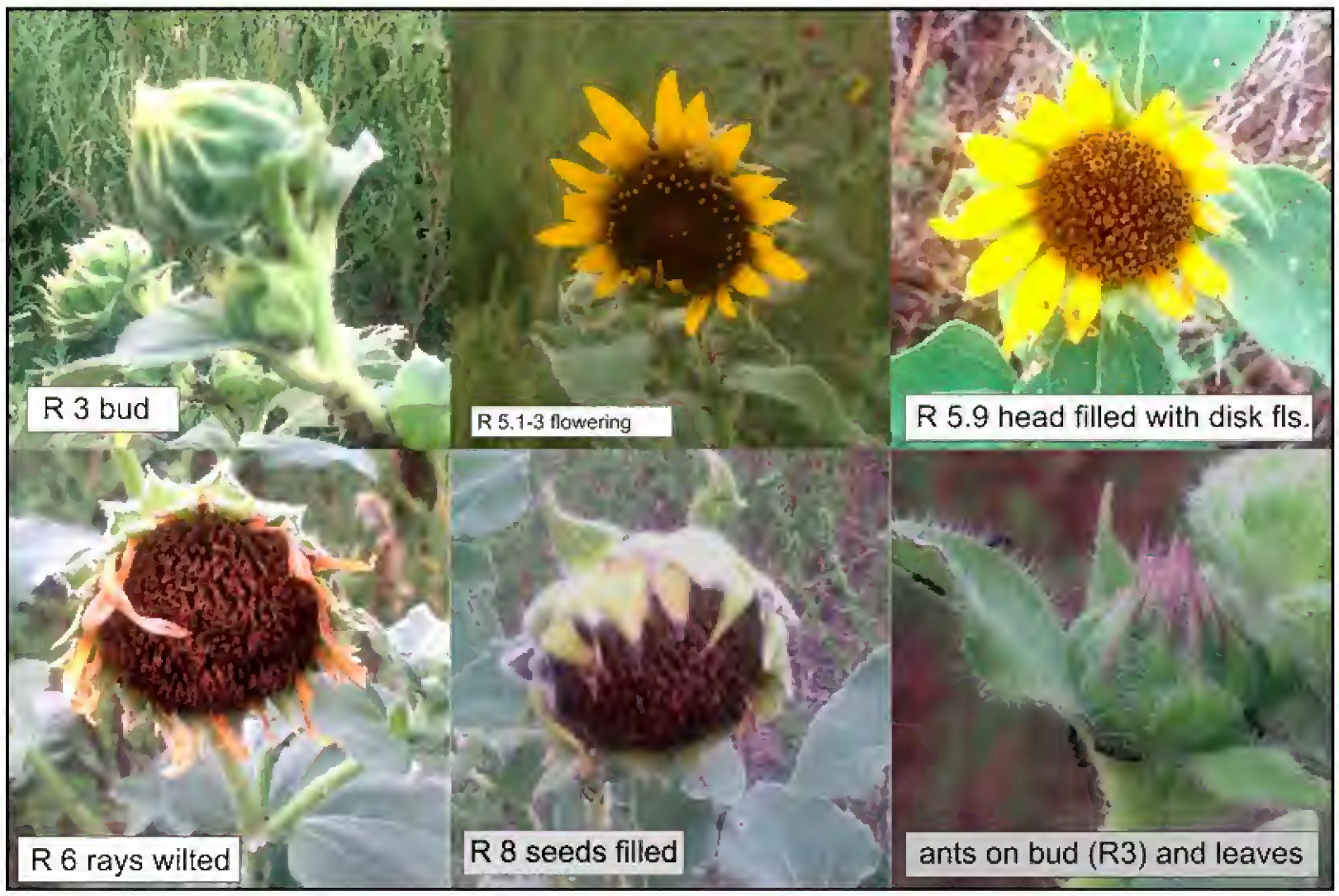

Figure 1. Growth stages of wild (*H. annuus*) sunflowers, Gruver, TX. Note black ants on the bud and leaves in lower right photo (from Adams et al. 2016). Sunflower growth stages termination is from Schnetter and Miller(1981).

Leaves were ground in a coffee mill (1mm). 3 g of air dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml pentane, the jar sealed, then placed on an orbital shaker for 18 hr. The pentane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan and the pentane evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

RESULTS

The yields of hydrocarbons (HC) by population are given in Table 1. The highest yield (8.60%) was from Gruver, TX followed by Lake Tanglewood, TX (8.47%) in the Texas Panhandle. The lowest yield was from Woodward, OK (2.62%) and Eagle Nest, NM (2.62%) followed by cultivated sunflowers (Oslo, TX)(3.20%). The Woodward population had smooth leaves as found in cultivated sunflowers. The plants and leaves were very large, although the heads were small. It appears that the Woodward population was a product of crosses between native and cultivated sunflowers and this resulted in the very low oil yield.

To visualize the variation in HC yields, the means were contour mapped (Fig. 2). Notice that the highest yields are in the Texas Panhandle. The lowest yields are in the west (EN, AZ, RO) and just off the caprock, east of the Texas Panhandle (PT, QN). The low yield at the WO (Woodward, OK) is in a population that is likely of hybrid origin between native and cultivated sunflowers. The southern Idaho - northern Utah area had medium-high yields. Of interest are the four populations near Waco, TX (MC, FC, LC, HC) that have 6.2, 5.3, 3.6, and 4.9% yields in a very small area. At this time, it is not known if

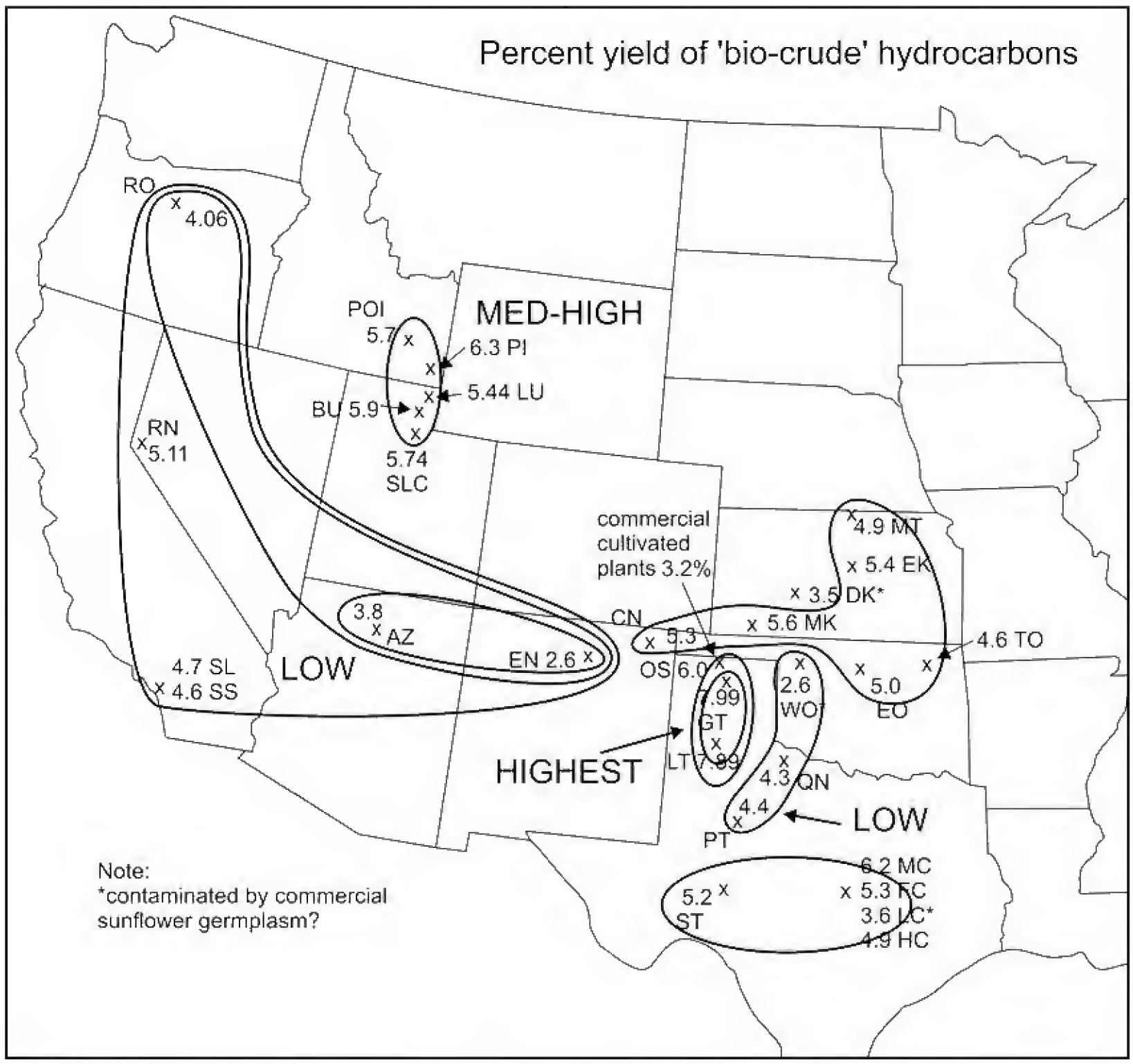

Figure 2. Geographic variation in % yields of HC by population. The asterisk (*) at the WO population indicates that the population is likely of hybrid origin between native and cultivated sunflowers. Note the low yield from a commercial sunflower field near Oslo, TX (lower left). See text for discussion.

the variation in yields is due to genetics or the environment. It is interesting that the correlation between % yield and leaf weight as only r= 0.18 (highly significantly different from zero, df = 327). But the correlation accounts for only 3.24% (r^2) of the variance. Thus, breeding for both increased % yields of HC and biomass seems feasible.

The variability of yields by population is mapped in Figure 3. Population variability in HC yields varied geographically and also between adjacent populations, suggesting the micro-habitat environments are important as well as limited genetic population size. One of the least variable populations was Pocatello, ID (POI, Fig. 3). This was a population of perhaps 50 plants, growing next to the sidewalk at an on-ramp to I15. It seems likely that POI is very inbred. The Brigham City, UT (BU) population, in a disturbed vacant lot where a new mall was recently built, was much more variable (Fig. 3). BU contained perhaps 100 plants, but a more extensive group of sunflowers grew nearby.

Clearly the most unusual situation was the Waco, TX area where 4 nearby populations (MC, FC, LC, HC, Fig. 3) showed very small to large amounts of variation in their HC yields. MT (Montrose, KS) was from only 3 cultivated plants raised from seed, so its small variability may be just chance.

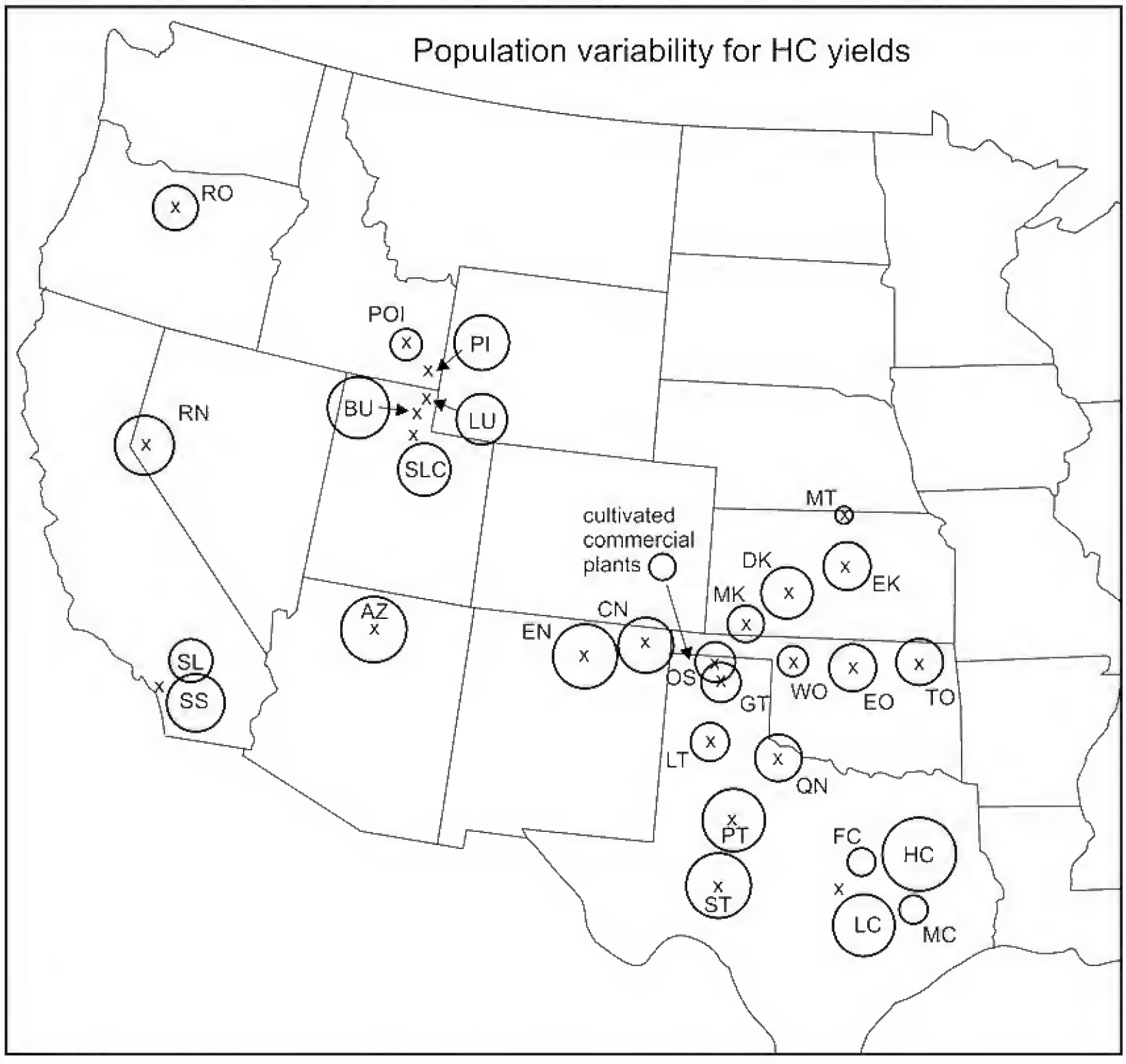

Figure 3. Population variability (coefficient of variation in HC yields) for the 29 populations sampled. The size (diameter) of the circles is proportional to their coefficient of variation.

The total yields of HC per the weight of 8 mature leaves is a measure of the grams of HC per plant (likely larger than if the entire plant were extracted). In Table 1, the yields range from 0.114 g/ 8 mature leaves (Eagle Nest, NM) to 1.428 g (Gruver, TX). Variation in yields shows (Fig. 4) the highest yields were in the Texas Panhandle (1.20 g - 1.23 g) and Ellsworth, KS (1.0 g) and Enid OK (0.88 g). The lowest yields were in the southwestern United States. Note the difference between the San Diego, large leaves (SL, .43 g) and small leaves (SS, .27 g). These are plants collected from the same population. Recall that the % yields were quite similar (Table 1, SS, 4.59%; SL, 4.68%).

The four populations near Waco, TX are quite variable and yields ranged from 0.26 g to 0.74 g. Whether this is due to micro-habitat environments or genetically isolated populations is not known at this time.

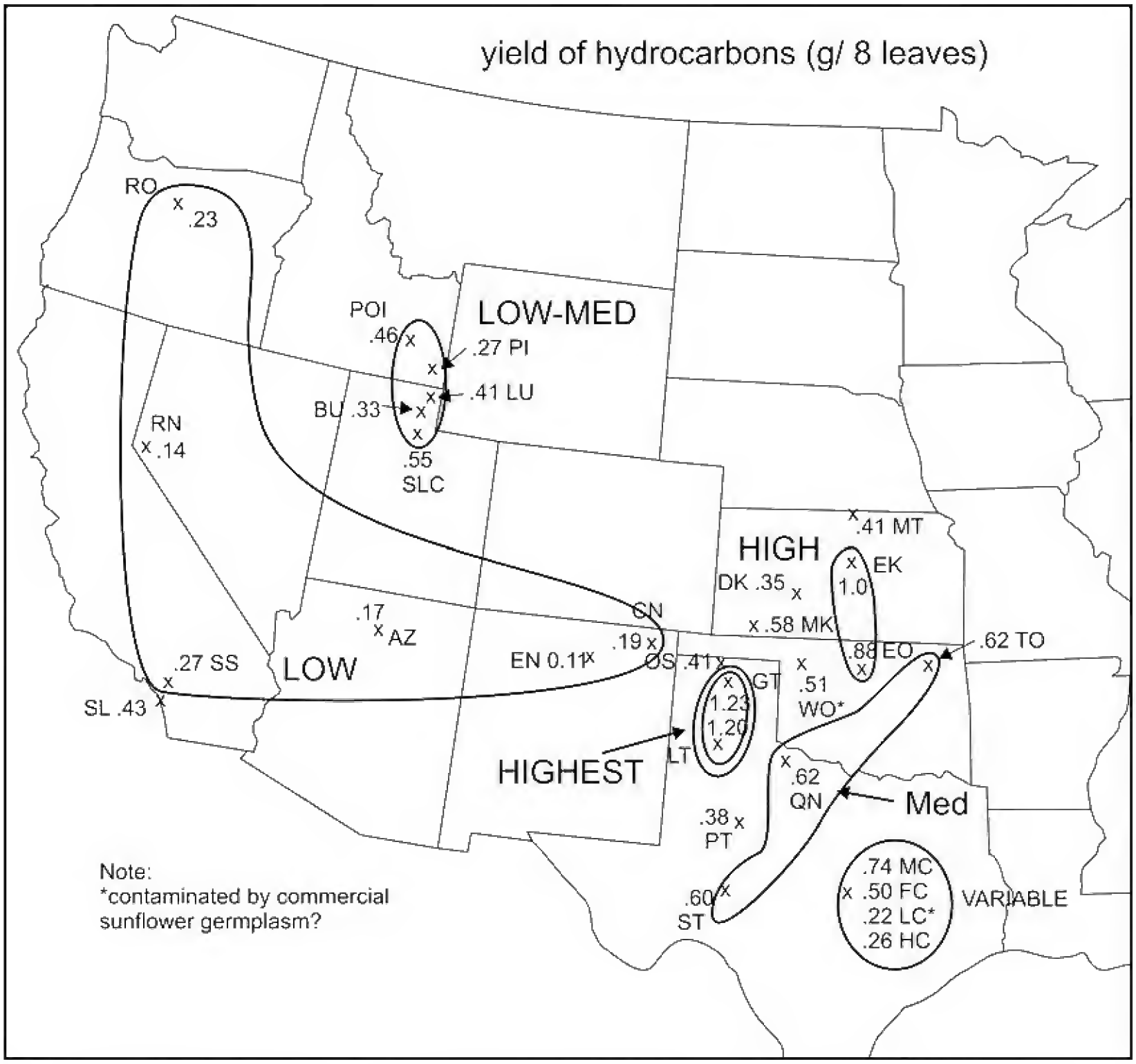

Figure 4. Geographic variation in the HC yields (g/ weight of 8 mature, dried leaves, basis).

The frequency distribution (329 individuals, Fig. 5) shows yields ranged from 1.0 to 12.63% with a skewed, normal distribution, and tailing towards the highest yielding plants (Fig. 5). The mean was 5.33%, with the top 5% being larger than a 8.7% yield. Seed from high yielding plants have been collected in preparation to examine genetic and environmental factors.

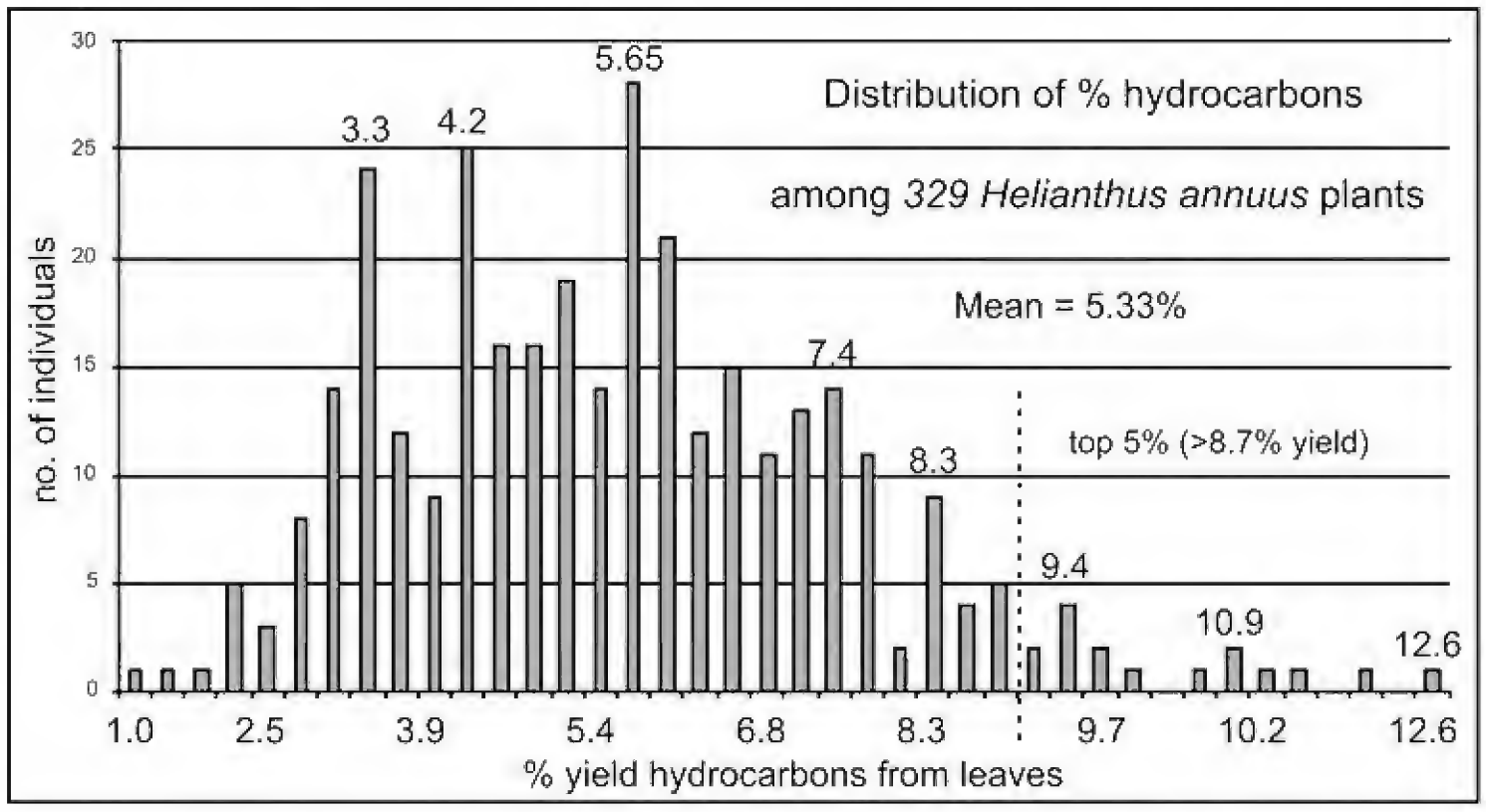

Figure 5. Frequency distribution of HC yields for 329 *H. annuus* plants. See text for discussion.

This study revealed the range of variation in native sunflowers is quite large, from 1.0 to 12.63%. It is remarkable to find such a wide range, but indicates the potential of *H. annuus* to produce copious amounts of hydrocarbons for use as fuel and in the petro-chemical industry. Many of the highest yielding plants were severely eaten by grasshoppers and covered with black (sugar) ants, feeding on resin extruded from the stem, petioles and leaf bracts. It could be that the high yields were responses to insect damage that induced defense chemicals. The induction of chemical defenses will be examined in a subsequent study, along with study of the effects of genetics vs. the environment on the production of hydrocarbons.

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Table 1. Yields of hydrocarbons (HC) *H. annuus*, from natural populations. Coefficient of variation computed as standard deviation / mean.

popn id, sample ids	population sampled	weight 8 lvs	% yield corr'd*	Coef. of variation	Range of yields	yield g/8 lvs
PT P1 - P0	14935 Post, TX	8.83	4.36	0.317	(2.33,6.63)	0.385
QN Q1-Q0	14936 Quanah, TX	14.41	4.32	0.238	(2.88,5.62)	0.623
MK M1-M0	14939 Meade, KS	10.38	5.64	0.186	(3.91,7.21)	0.585
DK D1-D0	14940 Dodge City, KS	9.75	3.52	0.262	(2.68,5.49)	0.346
EK E1-E0	14941 Ellsworth, KS	18.74	5.38	0.240	(3.63,7.35)	1.008
TO T1-T0	14942 Tulsa, OK	13.64	4.56	0.236	3.16,6.04)	0.622
EO O1-OT	14943 Enid, OK	17.60	4.97	0.239	(3.23,7.55)	0.875
WO W1-W0	14944 Woodward, OK, very large, smooth leaves	19.64	2.62	0.155	(1.92-3.09)	0.515
ST S1-S0	14945 grown from seed, ex Sonora, TX, PI413168	11.47	5.19	0.329	(1.99-7.55)	0.595
OS O1-TO	14946 Oslo, TX, native in prairie grass	6.75	5.95	0.203	(4.19-8.17)	0.406
	14947 Lake Tanglewood, TX					
LT: L1-L0	7/12/16, 1st collection	16.65	8.47	0.222	(6.73-12.15)	1.410
L2: LA-LJ	7-20-16, 2nd collection	13.92	**7.31**	0.163	(5.63-9.06)	1.018
			7.89 avg	0.195 avg	(5.63-12.15)	1.214
ID I1-I9	14948 grown from seed, ex Idaho, PI 531028	2.77	3.23	0.432	(1.0-6.14)	0.089
SS SA-SJ	14950 San Diego, CA, small leaves	5.83	4.59	0.292	(2.75-7.51)	0.268
SL SK-ST	14951 San Diego, CA, large leaves	9.04	4.68	0.218	(2.75-6.11)	0.432
	14952 Gruver, TX					
GT1:G1-G0	GT1 1-10 1 mi south	16.93	7.26	0.244	(5.01-11.06)	1.229
GT2:GA-GJ	GT2 11-20 1mi south	18.03	7.92	0.198	(6.25-10.78)	1.428
GT3:GKGT	GT3 21-30, 2 mi E, Rodeo	12.50	8.16	0.164	(7.00-10.51)	1.020
GT4:A1-AT	GT4 31-40, 1 mi south	14.50	8.60	0.235	(6.52-12.63)	1.247
			7.99 avg	0.201 avg	(5.01-12.63)	1.231
SC 1O-6O	14953 cultivated sunflower crop, Slough Farm, Oslo, TX	12.41	3.20	0.134	(2.75-3.85)	0.397
MC 1M-0M	14976 McLennan Co., TX *Holmes 16654*	11.95	6.18	0.144	(4.74-7.76)	0.739
FC 1F-0F	14977 Falls Co., Satin, TX *Holmes 16656*	9.49	5.29	0.142	(4.19-6.59)	0.502
LC 1L-0L	14978 Limestone Co. Mt. Calm, TX Holmes *16658*	6.13	3.58	0.313	(2.61-6.25)	0.219
HC 1H-0H	14979 Hill Co., TX *Holmes 16661*	5.21	4.92	0.372	(2.61-8.65)	0.263
EN 1E-0E	14980 Eagle Nest, NM	4.34	2.62	0.326	(1.17-3.85)	0.114
LU U1-U0	15023 Logan, UT	7.57	5.44	0.257	(3.98-8.67)	0.412
PI 1P-0P	15024 Preston, IT	4.29	6.30	0.278	(3.91-9.34)	0.270
POI 1I-0I	15025 Pocatello, ID	7.99	5.71	0.160	(4.46-7.55)	0.456
SLC 1U-0U	15026 Mill Creek, Salt Lake City, UT	9.54	5.74	0.266	(3.91-8.22)	0.548
RO R1-R0	15027 Redmond, OR	5.74	4.06	0.226	(3.37-6.28)	0.233
CN 1C-0C	14981 Capulin, NM	3.51	5.29	0.280	(3.16-8.34)	0.186
MT MA-MC	14982 grown from seed ex Montrose, KS, PI 413033	8.44	4.91	0.089	(4.65-5.42)	0.414

popn id, sample ids	population sampled	weight 8 lvs	% yield corr'd*	Coef. of variation	Range of yields	yield g/8 lvs
AZ Z1-Z0	15021 Camp Verde, AZ	4.48	3.79	0.332	(1.72-5.56)	0.170
BU B1-B0	15022 Brigham City, UT	5.70	5.90	0.312	(2.90-8.31)	0.336
RN R1-R0	15029 Reno, NV	2.87	5.11	0.299	(2.89-7.90)	0.142

*correction factor = soxhlet, 6hr extraction/ pentane 18 hr shaker yield = 2.06

Appendix I Population locations.

Helianthus petiolaris

common along roadside in sandy soil. flowering. 8.3 mi SW of Fritch, TX on TX 136, 35° 31' 53" N, 101° 38' 31" W. 3360 ft, Date: 4 June 2016, County: Potter; State: TX
Coll. Robert P. Adams No. 14937

Helianthus annuus L. below:

common along railroad and roadside in sandy soil. flowering. 5.3 mi SE of Post TX on US 84, 33° 01' 53" N, 101° 11' 25" W, 2300 ft, Date: 4 June 2016 County: Garza; State: TX
Coll. Robert P. Adams No. 14935

common along fence row and roadside in sandy soil. flowering.7 mi SE of Quanah, TX on US 287,34° 15' 57" N, 99° 36' 46" W, 1450 ft, Date: 5 June 2016 County: Hardeman; State: TX
Coll. Robert P. Adams No. 14936

1.5 mi s of Meade, on KS23, low area in edge of wheat field, 100s of plants in population, but generally uncommon. ~5% flowering. 37° 15' 49" N, 100° 20' 40" W, 2433 ft, Date: 7 July 2016; County: Meade; State: KS
Coll. Robert P. Adams No. 14939

8.5 mi NE of Dodge City, US 50, several on dirt piles of highway dept., but generally uncommon. ~5% flowering, 37° 47' 06" N, 99° 53' 14" W. 2534 ft. Date: 7 July 2016, County: Ford; State: KS
Coll. Robert P. Adams No. 14940

1.6 mi e of Ellsworth on KS140, on fence row on s side of wheat field, 20 plants, but generally uncommon. ~10% flowering. 38° 44' 24" N, 98° 11' 53" W, 1600 ft, Date: 7 July 2016, County: Ellsworth; State: KS
Coll. Robert P. Adams No. 14941

15 plants on disturbed area next to South Ash St. (just south of OK364), but generally uncommon, Jenks, OK (sw suburb of Tulsa). ~5% flowering. 36° 00' 57.85" N, 95° 58' 07.61" W, 613 ft, Date: 9 July 2016, County: Tulsa; State: OK
Coll. Robert P. Adams No. 14942

5.5 mi e of Enid on OK412,on fence row, side of wheat field, few plants but generally uncommon. ca 5% flowering, ~5% flowering. 36° 23' 51" N, 97° 46' 51" W, 1160 ft., Date: 9 July 2016, County: Garfield; State: OK
Coll. Robert P. Adams No. 14943

smooth leaves! 2.8 mi e of Woodward on OK412,on fence row, side of grass field, few plants but generally uncommon. ca 5% flowering mostly pre-flowering. 36° 25' 53" N, 99° 20' 28" W, 1880 ft., Date: 9 July 2016, County: Woodward; State: OK
Coll. Robert P. Adams No. 14944

cultivated at Oslo, TX, from seed (USDA PI413168-NC7) ex Sonora, TX. 80% flowering, 36° 25' 12.3" N, 101° 31' 54.6" W, 3239 ft, Date: 12 July 2016, County: cult in Hansford; State: TX.
Coll. Robert P. Adams No. 14945

native in grassland, JP & Amy TeBeest farm, 1 mi. s of Oslo Lutheran Church. ~5% flowering. 36° 25' 12.3" N, 101° 31' 54.6" W, 3239 ft., Date: 12 July 2016, County: Hansford; State: TX
Coll. Robert P. Adams No. 14946

2- 3 ft plants, lots of resin on petioles and leaf veins, many sugar (black) ants, most with wilted leaves, very dry in July, common in native grass and on disturbed roadside, brush dump area, Lake Tanglewood, ~50% flowering, 35° 04' 23.7" N, 101° 47' 29.0" W, 3239 ft., Date: 12 July 2016, County: Randall; State: TX
Coll. Robert P. Adams No. 14947

cultivated at Oslo, TX, from seed (USDA PI 531028) ex Idaho, 80% flowering.36° 25' 12.3" N, 101° 31' 54.6" W, 3239 ft, Date: 12 July 2016, County: cult in Hansford; State: TX
Coll. Robert P. Adams No. 14948

plants 2' tall, with small leaves, along San Pasqual Rd, 33° 05' 08.2" N, 117° 01' 46.2" W, 353 ft.
Date: 6 July 2016, County: San Diego; State: CA, Coll. Jim A. Bartel 1636
Lab Acc. Robert P. Adams No. 14950

plants to 8' tall, with large leaves, along San Pasqual Rd, 33° 05' 08.2" N, 117° 01' 46.2" W, 353 ft/ Date: 8 July 2016, County: San Diego; State: CA, Coll. Jim. Bartel 1636
Lab Acc. Robert P. Adams No. 14951

2-3' tall, 10% flowering, lots of damage to leaves by grasshoppers, etc., some with many black (sugar) ants, copious resin at base of leaves, along fence row, on TX 206, 1-5:1.2 mi s, 6-10: 1.3 mi. s of Gruver, TX. 36° 14' 52" N, 101° 24' 52" W, 3161 ft, Date: 16 July 2016, County: Hansford; State: TX
Coll. Robert P. Adams No. 14952

cultivated, irrigated near Oslo, TX, on Slough farm. at R-5.1 stage. 36° 22' 42.17" N, 101° 37' 21.4" W, 3350 ft., leaves mostly smooth. Date: 17 July 2016, County: cult in Hansford; State: TX
Coll. Robert P. Adams No. 14953

Coll. Walter Holmes
(WCH16654) McLennan Co. 12th Street at Flat Creek, Robinson (Waco), 27 July 2016 , Walter Holmes
Lab Acc. Robert P. Adams 14976
(WCH 16656) Falls Co. near Satin on FR 434, prairie roadside, 28 July 2016, Walter Holmes
Lab Acc. Robert P. Adams 14977
(WCH 16658) Limestone Co. near jct of Limestone Co roads 102 and 106, south of Mt. Calm, prairie, 29 July 2016, Walter Holmes
Lab Acc. Robert P. Adams 14978
(WCH 16661) Hill Co. US Hwy 84, West of Mt. Calm near jct with West Somers Lane, 29 July 2016, Walter Holmes
Lab Acc. Robert P. Adams 14979

roadside waste area, Eagle Nest, NM, 36° 33.650' N, 105° 15.969' W, 8260 ft, Date: 8 Aug 2016, County: Colfax; State: New Mexico, Coll. Amy TeBeest
Lab acc. Robert P. Adams 14980

roadside waste area, Capulin (city), NM, some grasshopper damage, 36° 44.527' N, 104° 00.178' W, 6820 ft, Date: 8 Aug 2016, County: Union: State: New Mexico, Coll. Amy TeBeest
Lab acc. Robert P. Adams 14981

cultivated at Oslo, TX, from seed (USDA PI1413033), ex Montrose, KS. Date: 2 Aug 2016, Coll. Amy TeBeest,
Lab acc. Robert P. Adams 14982

along roadsides. 16-18 mi east of Camp Verde on AZ 260. 34.489° N, 111.597° W, 5900 ft, Date: Aug. 27, 2016, County: Yavapai; State: AZ, Coll. David Thornburg ns,